

Sprouts of the broccoli cultivar Everest contained 130-fold more inducer potential (units/g fresh weight) than mature vegetables. The inducer activity in broccoli was significantly higher than in daikon.

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*Example 5**INDUCER POTENTIAL OF BROCCOLI SPROUT EXTRACTS*

Inducer potential of a series of water extracts of 3-day old broccoli sprouts of the cultivar Saga were determined. Plants were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga by a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite containing approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm² for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

Plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts (approximately 25 mg fresh wt/sprout) were gently harvested and immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. The sprouts were then either strained from the boiled infusion [tea, soup] or homogenized in it, and the residue then removed by filtration or centrifugation.

Data in Table 3 represent both homogenates and infusions. Preparations were stored at -20°C until assayed. Inducer potential of plant extracts, prepared

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as described above, was determined as described in Definitions section above.

TABLE 3
Inducer Potentials of Hot Water Extracts
of 3-Day Saga Broccoli Sprouts

EXTRACT NO.	units/g fresh weight
1	500,000
2	370,000
3	455,000
4	333,000
5	435,000
6	333,000
7	625,000
8	250,000
9	313,000
10	357,000
11	370,000
12	370,000
13	217,000
14	222,000
15	1,000,000
16	714,000
17	435,000
18	1,250,000
19	263,000
AVERAGE	464,000 \pm 61,600 S.E.M.

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Some variability in the amount of Phase 2 enzyme-inducer potential was detected. High levels of Phase 2 enzyme-inducer potential, however, were consistently observed.

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Example 6

**HOT WATER BROCCOLI EXTRACTS TREATED
WITH DAIKON MYROSINASE**

QR activity in a hot water broccoli extract increased in the presence of a vegetable source of myrosinase. An aqueous extraction of 3-day old sprouts of broccoli cultivar Saga grown on water agar, in which myrosinase was inactivated by boiling for 3 min, was divided into 6 different 150 ml aliquots. Nine-day old daikon sprouts, a rich source of the enzyme myrosinase, were added to this cooled infusion in amounts equivalent to 0, 5, 9, 17, 29 and 40% (w/w) of the broccoli. QR activity, as determined in the Definition section, of the control extracts containing 0% daikon was 26,300 units/gram fresh weight while QR activity of the extracts that had received daikon as a source of myrosinase ranged from 500,000 to 833,000 units/gram fresh weight of broccoli. Accordingly, myrosinase present in the daikon sprouts, increased the QR activity in the broccoli extract greater than 19-fold.

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Example 7

**GLUCORAPHANIN AND GLUCOERUCIN ARE THE PREDOMINANT
GLUCOSINOLATES IN HOT WATER EXTRACTS OF BROCCOLI
(CULTIVAR SAGA) SPROUTS**

Paired Ion Chromatography (PIC). Centrifuged hot water extracts of 3-day-old broccoli (cultivar Saga) sprouts were subjected to analytical and preparative PIC on a reverse phase C18 Partisil ODS-2 HPLC column in ACN/H₂O (1/1, by vol.) with tetraoctylammonium (TOA) bromide as the counter-ion. Only three well-separated peaks were detected: peak A eluted at 5.5 min, B at 11.5

min, and C at 13 min at a molar ratio [A:B:C] of ca. 2.5 : 1.6 : 1.0 (monitored by UV absorption at 235 nm), and they disappeared if the initial extracts were first treated with highly purified myrosinase. Peaks A, B, and C contained no significant inducer activity, and cyclocondensation assay of myrosinase hydrolysates showed that only Peaks A and C produced significant quantities of isothiocyanates, accounting for all the inducer activity. See Zhang et al., *Anal. Biochem.* 205: 100-107 (1992). Peak B was not further characterized. Peaks A and C were eluted from HPLC as TOA salts but required conversion to ammonium salts for successful mass spectroscopy, NMR and bioassay. The pure peak materials were dried in a vacuum centrifuge, redissolved in aqueous 20 mM NH₄Cl, and extracted with chloroform to remove excess TOA bromide. The ammonium salts of glucosinolates remained in the aqueous phase, which was then evaporated.

Identification of Glucosinolates. The ammonium salts of Peaks A and C were characterized by mass spectrometric and NMR techniques: (a) negative ion Fast Atom Bombardment (FAB) on a thioglycerol matrix; this gave values of 436 (Peak A) and 420 (Peak C) amu for the negative molecular ions, and (b) high resolution NMR, as shown in Figure 2, provided unequivocal identification of the structure. Peak A is glucoraphanin [4-methylsulfinylbutyl glucosinolate], and Peak C is the closely related glucoerucin [4-methylthiobutyl glucosinolate]. These identifications and purity are also consistent with the inducer potencies; Peaks A and C, after myrosinase hydrolysis had potencies of 36,100 and 4,360 units/ μ mol, respectively, compared with reported CD values of 0.2 μ M (33,333 units/ μ mol) for sulforaphane and 2.3 μ M (2,900 units/ μ mol) for erucin. CD values are the concentrations of a compound required to double the QR specific activity in Hepa 1c1c7 murine hepatoma cells. Since there are no other glucosinolate peaks, and the inducer activity of peak A and C account for the total inducer activity of the extracts, it is

therefore likely that in this cultivar of broccoli, there are no significant quantities of other inducers, i.e., no indole or hydroxyalkenyl glucosinolates. Further, the isolated compounds are therefore substantially pure.

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Example 8

**COMPARISON OF AQUEOUS AND ORGANIC SOLVENT TECHNIQUES
FOR EXTRACTION OF INDUCER POTENTIAL**

Plants were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga, with 70% ethanol followed by 1.3% sodium hypochlorite and 0.001%alconox. The seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm² for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity, and temperature control (16 hours light, 25°C/8 hours dark, 20°C).

The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A portion of the plants was homogenized with 10 volumes of the DMF/ACN/DMSO solvent at -50°C, as described in Example 1, which dissolves nearly all the non-lignocellulosic plant material. Alternatively, the bulk of the harvested plants was plunged into 5 volumes of boiling water for 3 min to inactivate endogenous myrosinase and to extract glucosinolates and isothiocyanates. The cooled mixture was homogenized, centrifuged, and the supernant fluid was stored at -20°C.

Inducer potential of plant extracts, prepared by the two methods described above, was determined by the microtiter plate bioassay as described above. Typical inducer potentials in an average of 5 preparations were 702,000 (DMF/ACN/DMSO extracts) and 505,000 (aqueous extracts) units/g fresh weight of sprouts.

Spectrophotometric quantitation of the cyclocondensation product of the reaction of isothiocyanates with 1,2-benzenedithiole was carried out as described in Zhang et al., *Anal. Biochem.* 205: 100-107 (1992). Glucosinolates were rapidly converted to isothiocyanates after addition of myrosinase. About 6% of the total hot water extractable material [dissolved solids] consisted of glucosinolates. These results demonstrate that (a) isothiocyanate levels in the crude plant extracts are extremely low; (b) myrosinase rapidly converts abundant glucosinolates to isothiocyanates; (c) hot water extraction releases over 70% of the inducer activity extractable with a triple solvent mixture permitting recovery of most of the biological activity in a preparation that is safe for human consumption; and (d) over 95% of the inducing potential in the intact plant is present as glucosinolates and therefore no other inducers are present in biologically significant quantities.

Example 9

20 DEVELOPMENTAL REGULATION OF GLUCOSINOLATE PRODUCTION

Preliminary experiments in which field grown broccoli (cultivar DeCicco) was harvested at sequential time points from the same field indicated that on a fresh weight basis, inducer potential declined from the early vegetative stage through commercial harvest, but appeared to increase at late harvest (onset of flowering). These data suggested that inducer potential might be highest in seeds. Subsequent studies have shown that when seeds of 8 broccoli cultivars were surface sterilized and grown under gnotobiotic conditions, Phase 2 enzyme-inducer potential was highest in seeds and declined progressively (on a fresh weight basis) over time throughout the first 14 days of seedling growth.

Expressed on a per plant basis, however, activity remained constant over this period, suggesting that at

this early stage of growth there was no net synthesis of glucosinolates. However, when the glucosinolate profiles of market stage broccoli heads and 3 day old sprouts (cultivar Emperor) were compared, there was a profound difference in the apparent glucosinolate compositions of these plants.

Sprouts were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Emperor with a 1 minute treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm² for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled; broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

Plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts [approximately 25 mg fresh wt/sprout], were gently harvested and immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. The sprouts were then strained from the boiled infusion [tea, soup] and the infusion was stored at -20°C until assayed.

Market stage heads were obtained by germinating seeds of the same seedlot in a greenhouse in potting soil, transplanting to an organically managed field in Garrett County, MD and harvested at market stage. Heads were immediately frozen upon harvest, transported to the laboratory on ice and extracts were prepared in an identical fashion to those described above for sprouts

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except that approximately 3 gram floret tissue samples were used for extraction.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described in Example 1. Paired ion chromatography revealed two major peaks, probably glucobrassicin and neo-glucobrassicin, in extracts of market stage heads with similar retention times to glucobrassicin (indole-3-ylmethyl glucosinolate) and neo-glucobrassicin (1-methoxyindole-3-ylmethyl glucosinolate). This observation is consistent with published reports on the glucosinolate composition of mature broccoli plants. However, paired ion chromatography under the same conditions of identically prepared extracts of 3-day-old sprouts showed absence of glucobrassicin or neo-glucobrassicin. Additionally, 3-day-old sprouts of different broccoli cultivars produce different mixtures of glucosinolates. Accordingly, glucosinolate production is developmentally regulated.

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Example 10
EVALUATION OF ANTICARCINOGENIC ACTIVITIES
OF BROCCOLI SPROUT PREPARATIONS IN THE HUGGINS
DMBA (9,10 DIMETHYL-1,2-BENZANTHRACENE)
MAMMARY TUMOR MODEL

Sprouts were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga with a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm² for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A large quantity of sprouts was harvested by immediately and rapidly plunging into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase, as well as extracting glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. Sprouts were then strained from the boiled infusion [tea, soup] and the infusion was lyophilized and stored as a dry powder at -20°C [designated Prep A]. Other sprouts, similarly prepared were extracted with boiling water, cooled to 25°C and were amended with a quantity of 7 day old daikon sprouts equivalent to approximately 0.5% of the original fresh weight of broccoli sprouts. This mixture was homogenized using a Brinkman Polytron Homogenizer and incubated at 37°C for 2 hours following which it was filtered through a sintered glass filter, lyophilized as above and stored as a dried powder at -20°C [designated Prep B].

QR inducer activity and inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. The induction of QR activity in preparation A is largely due to glucosinolates; predominantly glucoraphanin, which is the glucosinolate of sulforaphane, but this preparation also contains some glucoerucin, which is the sulfide analog of glucoraphanin. The induction QR activity of preparation B is almost exclusively due to isothiocyanates arising from treatment of glucosinolates with myrosinase.

Female Sprague-Dawley rats received at 35 days of age were randomized; 4 animals per plastic cage. All animals received 10 mg DMBA, by gavage in 1 ml sesame oil, at age 50 days. Sprout preparations (A or B) or vehicle control were given by gavage at 3, 2 & 1 day prior to DMBA, on

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the day of DMBA (2 hr prior to the DMBA dose) and on the
day following DMBA dosing. The vehicle used was 50%
Emulphor 620P / 50% water. Animals were maintained on a
semi-purified AIN-76A diet *ad libitum* from the time of
5 receipt until termination of the experiment (167 days of
age).

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TABLE 4

**ANTICARCINOGENIC ACTIVITIES OF BROCCOLI SPROUT EXTRACTS
IN THE DMBA RAT MAMMARY TUMOR MODEL**

GROUP	TREATMENT	NUMBER OF ANIMALS AT TERMINATION	TOTAL TUMOR NUMBER	MULTIPLICITY: NUMBER OF TUMORS PER RAT
CONTROL	DMBA only	19	34	1.79
PREPARATION A (Glucosinolate)	324 mg/dose (100 μ mol sulforaphane equiv.)	18	19	1.05
PREPARATION B (Isothiocyanate)	424 mg/dose (100 μ mol sulforaphane equiv.)	20	11	0.55

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The development of palpable tumors was delayed for as much as 5 weeks by the administration of sprout extracts. Rats treated with either Preparation A or B had significantly fewer tumors than the untreated control, and the multiplicity of tumors (tumors per rat) was significantly lower in the animals receiving Preparations A or B.

Example 11

METABOLISM AND CLEARANCE OF GLUCOSINOLATES IN HUMANS

Two male, non-smoking volunteers ages 35 and 40 years, each in good health, were put on a low vegetable diet in which no green or yellow vegetables, or condiments, mustard, horseradish, tomatoes or papayas were consumed. After 24 hours on such a diet, all urine was collected in 8 hr aliquots. After 24 hours of baseline data, subjects ingested 100 ml of broccoli sprout soup (prepared as below), containing 520 μ mol of glucosinolates.

The sprouts were prepared by first surface sterilizing seeds of *Brassica oleracea* variety *italica* (broccoli) cultivar Saga with a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with ca. 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm² for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C). The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A large quantity of sprouts was harvested by immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as

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well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. Following the boiling step, sprouts were homogenized directly in their infusion water for 1 min using a Brinkman Polytron Homogenizer and the preparations were frozen at -79°C until use.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. Inducer potential is nearly all due to glucosinolates; predominantly glucoraphanin, which is the glucosinolate of sulforaphane, but some glucoerucin which is the sulfide analog of glucoraphanin was also present. When converted to isothiocyanates by the addition of purified myrosinase, Phase 2 enzyme-inducing potential was 100,000 units/ml and contained 5.2 μmol of isothiocyanates per ml, as determined by the cyclocondensation reaction described in Example 7. Thus, the subjects consumed a total of 520 μmol of glucosinolates.

Collection of 8 hour urine samples was continued for an additional 30 hours. Urinary excretion of isothiocyanate conjugates (dithiocarbamates) was monitored using the cyclocondensation reaction as described in Example 7.

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TABLE 5

**EXCRETION OF DITHIOCARBAMATES BY TWO SUBJECTS
INGESTING 520 MICROMOLES OF GLUCOSINOLATES
EXTRACTED FROM SAGA BROCCOLI**

TIME	CONDITION	SUBJECT 1	SUBJECT 2
Collection Time (hours)		μ mol Dithiocarbamate per 8 hour urine collection	
8	baseline	1.4	2.7
16	baseline	2.1	0.9
24	baseline	1.7	5.4
32	1st 8 hour post-dose	23.2	20.4
40	2nd 8 hour post-dose	9.9	36.8
48	3rd 8 hour post-dose	4.4	14.0
56	4th 8 hour post-dose	4.2	4.1
Total post-dose minus average baseline:		39.8	63.2
Total as Percent of dose:		6.7%	12.2%

The two subjects studied metabolically converted a significant fraction of the ingested glucosinolates to the isothiocyanates which were converted to cognate dithiocarbamates and measured in the urine.

Example 12

**EFFECTS OF PHYSICAL INTERVENTIONS ON SPROUT GROWTH
ON PRODUCTION OF INDUCERS OF QUINONE REDUCTASE**

Sprouts were prepared by first surface sterilizing seeds of *Raphanus sativum* (daikon) by a 1 minute treatment with 70% ethanol, followed by a 15 min treatment with 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown

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in sterile plastic containers at a density of approximately 8 seeds/cm² for 7 days on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light 25°C/8 hours dark, 20°C).

Treated sprouts were irradiated with germicidal UV light for 0.5 hr on days 5 and 6. Treated sprouts were only half the height of the untreated controls. Plants were harvested on day 7 by rapidly and gently collecting the plants from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts were harvested by immediate and rapid plunging into approximately 10 volumes of DMF/ACN/DMSO (1:1:1) at approximately -50°C in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothiocyanates. Sprouts were immediately homogenized with a ground glass mortar and pestle and stored at -20°C.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. Inducer potential of the UV-treated sprouts was over three times that of untreated controls. Treatment of sprouts with ultraviolet light therefore increased the Phase 2 enzyme-inducer potential of the plant tissue.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention, which is defined by the following claims. All publications and patent applications mentioned in this specification are indicative of the

What Is Claimed Is:

1. Cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts, harvested prior to the 2-leaf stage.
2. The cruciferous sprouts according to claim 1, wherein said sprouts are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemmifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.
3. The cruciferous sprouts according to claim 2, wherein said sprouts are a *Brassica oleracea* variety *italica*.
4. The cruciferous sprouts according to claim 1, wherein said sprouts are a *Brassica oleracea* variety *botrytis*.
5. The cruciferous sprouts according to claim 1, wherein said sprouts are a *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.
6. The cruciferous sprouts according to claim 1, wherein said sprouts are substantially free of Phase 1 enzyme-inducing potential.
7. A non-toxic solvent extract of the cruciferous sprouts according to claim 1.
8. The non-toxic solvent extract according to claim 7, wherein said solvent is water.
9. The non-toxic solvent extract according to claim 8, further comprising a cruciferous vegetable comprising an active myrosinase enzyme.

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10. The non-toxic solvent extract according to claim 9, wherein said cruciferous vegetable is of the genus *Raphanus*.

20. The non-toxic solvent extract according to claim 19, wherein said cruciferous vegetable is of the genus *Raphanus*.

21. A method of preparing a food product rich in glucosinolates, comprising germinating cruciferous seeds, with the exception of cabbage, cress, mustard and radish seeds, and harvesting sprouts prior to the 2-leaf stage, to form a food product comprising a plurality of sprouts.

22. The method according to claim 21, wherein said sprouts contain non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

23. The method according to claim 21, wherein said seeds are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemnifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

24. The method according to claim 23, wherein said seeds are *Brassica oleracea* variety *italica*.

25. The method according to claim 23, wherein said seeds are *Brassica oleracea* variety *botrytis*.

26. The method according to claim 25, wherein said seeds are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

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27. A food product rich in glucosinolates made by the process according to claim 21.

28. A method of preparing a food product, comprising extracting glucosinolates and isothiocyanates from cruciferous sprouts according to claim 1 with a non-toxic solvent, removing the extracted sprouts from said solvent, and recovering the extracted glucosinolates and isothiocyanates.

29. A method of preparing a food product according to claim 28, wherein active myrosinase enzyme is mixed with said cruciferous sprouts, or said extracted glucosinolates and isothiocyanates, or both said cruciferous sprouts or said extract.

30. A method of preparing a food product rich in glucosinolates, comprising germinating cruciferous seeds that produce sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and which contain non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, and harvesting sprouts prior to the 2-leaf stage to form a food product comprising a plurality of sprouts.

31. The method according to claim 30, wherein said seeds are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemma*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

32. The method according to claim 31, wherein said seeds are *Brassica oleracea* variety *italica*.

33. The method according to claim 31, wherein said seeds are *Brassica oleracea* variety *botrytis*.

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34. The method according to claim 33, wherein said seeds are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

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35. A food product rich in glucosinolates, made by the process according to claim 30.

36. A method of preparing a food product, comprising introducing cruciferous seeds, wherein said seeds produce sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, into another edible ingredient.

37. A method of preparing a food product, comprising extracting glucosinolates and isothiocyanates with a non-toxic solvent and isothiocyanates from cruciferous seeds, sprouts, plants or plant parts wherein seeds that produce said sprouts, plant, or plant parts, have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and wherein said seeds, sprouts, plants or plant parts have non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, and recovering the extracted glucosinolates and isothiocyanates.

38. A method of preparing a food product according to claim 37, wherein active myrosinase enzyme is mixed with said cruciferous seeds, sprouts or plants; or said extracted glucosinolates and isothiocyanates; or both said cruciferous seeds, sprouts or plants and said extract.

39. A method of reducing the level of carcinogens in a mammal, comprising administering to a mammal an

effective amount of cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts.

40. A method of reducing the level of carcinogens in a mammal, comprising administering to a mammal an effective amount of cruciferous sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

41. A method of extracting glucosinolates and isothiocyanates from plant tissue comprising the steps of homogenizing said plant tissue in an excess of a mixture of dimethyl sulfoxide, acetonitrile and dimethylformamide at a temperature sufficient to inactivate myrosinase enzyme activity.

42. A food product comprising cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts, harvested prior to the 2-leaf stage, cruciferous seeds; extracts of said sprouts or seeds; or any combination of said sprouts, seeds or extracts.

43. A method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the food product according to claim 42.

44. A food product comprising cruciferous sprouts harvested prior to the 2-leaf stage, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolate and goitrogenic hydroxybutenyl glucosinolates; cruciferous seeds; extracts of said sprouts or seeds; or any combination of said sprouts, seeds or extracts.

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45. A method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the food product according to claim 44.

46. Cruciferous sprouts harvested prior to the 2-leaf stage, wherein the ratio of monofunctional to bifunctional inducers is at least 20 to 1.

47. A food product supplemented with a purified or partially purified glucosinolate.

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Docket No. 46528/102/JOHO**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CANCER CHEMOPROTECTIVE FOOD PRODUCTS

the specification of which (check one)

- ☒ is attached hereto
- ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sanderoock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

Send all correspondence to FOLEY & LARDNER, 3000 K Street, N.W., Suite 500, Washington, DC 20007-5109. Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor Jed W. FAHEY	Signature of First or Sole Inventor <i>Jed W. Fahey</i>	Date 9/13/95
Residence Address 6704 RIDGE RD., ELDERSBURG, MD 21784	Country of Citizenship United States	
Post Office Address 6704 RIDGE RD., ELDERSBURG, MD 21784		

Signatures should conform to names as typewritten. ☒ Additional inventors on attached Page 2.

PAGE 2

Docket No. 46528/102/JOHQ

Full Name of Second Inventor Paul TALALAY		Signature of Second Inventor Paul Talalay	Date 9/13/95
Residence Address 5512 BOXHILL LANE, BALTIMORE MD 21210		Country of Citizenship United States	
Post Office Address 5512 BOXHILL LANE BALTIMORE MD 21210			

[illegible]

Applicant or Patentee: FAHEY et al.
 Serial or Patent No.: 08/528,838 Atty. Dkt. No. 46528/102/JOHO
 Filed or Issued: 9/15/95
 For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) AND 1.27 (c)) — NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Johns Hopkins School of Medicine
 ADDRESS OF ORGANIZATION: 2024 E. Monument Street, Suite 2-100, Baltimore, MD 21205
 TYPE OF ORGANIZATION:
☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) AND 501(c)(3))
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
 (NAME OF STATE)
 (CITATION OF STATUTE)
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) AND 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
 (NAME OF STATE)
 (CITATION OF STATUTE)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) or (b) of Title 35, United States Code with regard to the invention entitled CANCER CHEMOPROTECTIVE FOOD PRODUCTS by inventor(s) FAHEY et al. described in

☒ the specification filed herewith
☐ application serial no. _____, filed _____
☐ patent no. _____, issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: _____
 ADDRESS: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT CORPORATION

NAME: _____
 ADDRESS: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT CORPORATION

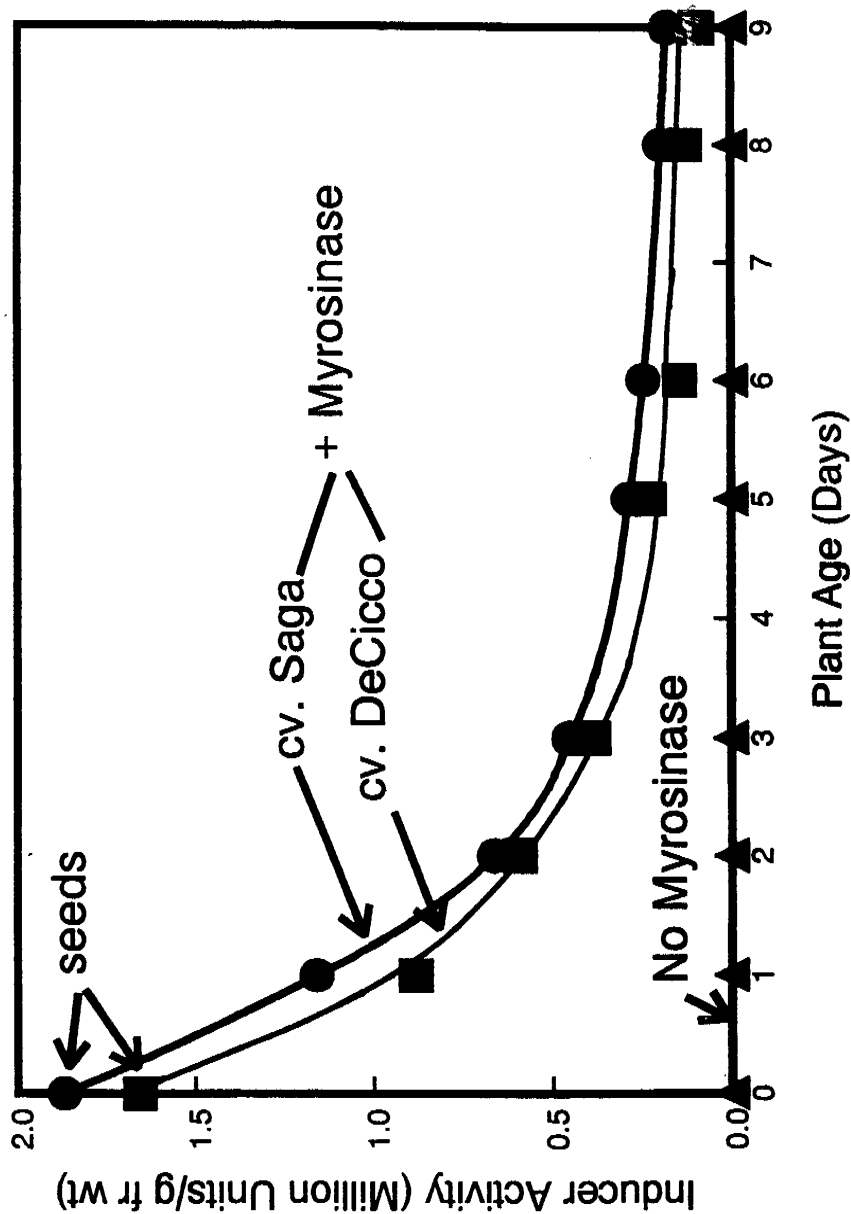
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate: (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: David A. Blake, Ph.D.
 TITLE OF PERSON OTHER THAN OWNER: Executive Vice Dean
 ADDRESS OF PERSON SIGNING: 3720 Rutland Avenue, Baltimore, Maryland 21205
 SIGNATURE: [Signature] DATE: 5/9/08

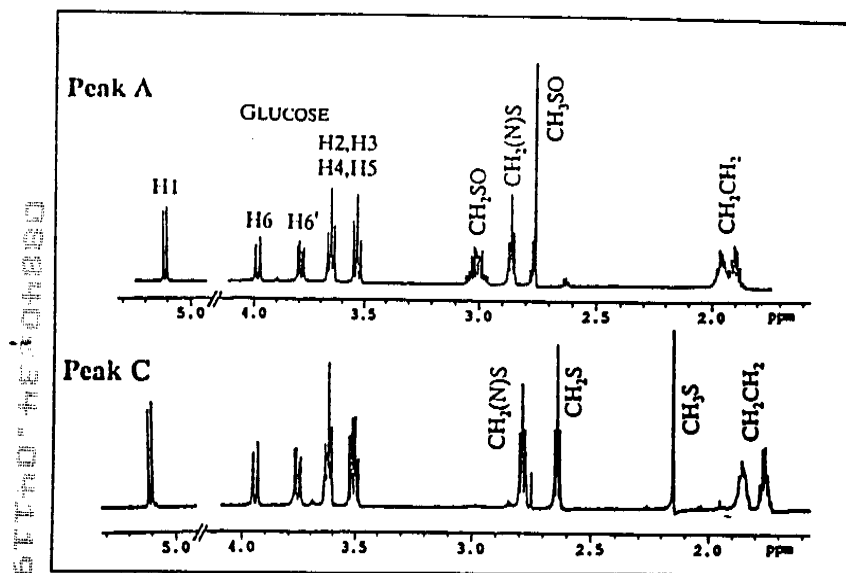
Inducer Activity of Broccoli Sprouts

Effect of Plant Age



▲ = < 1000 Units/g fr wt

Figure 1

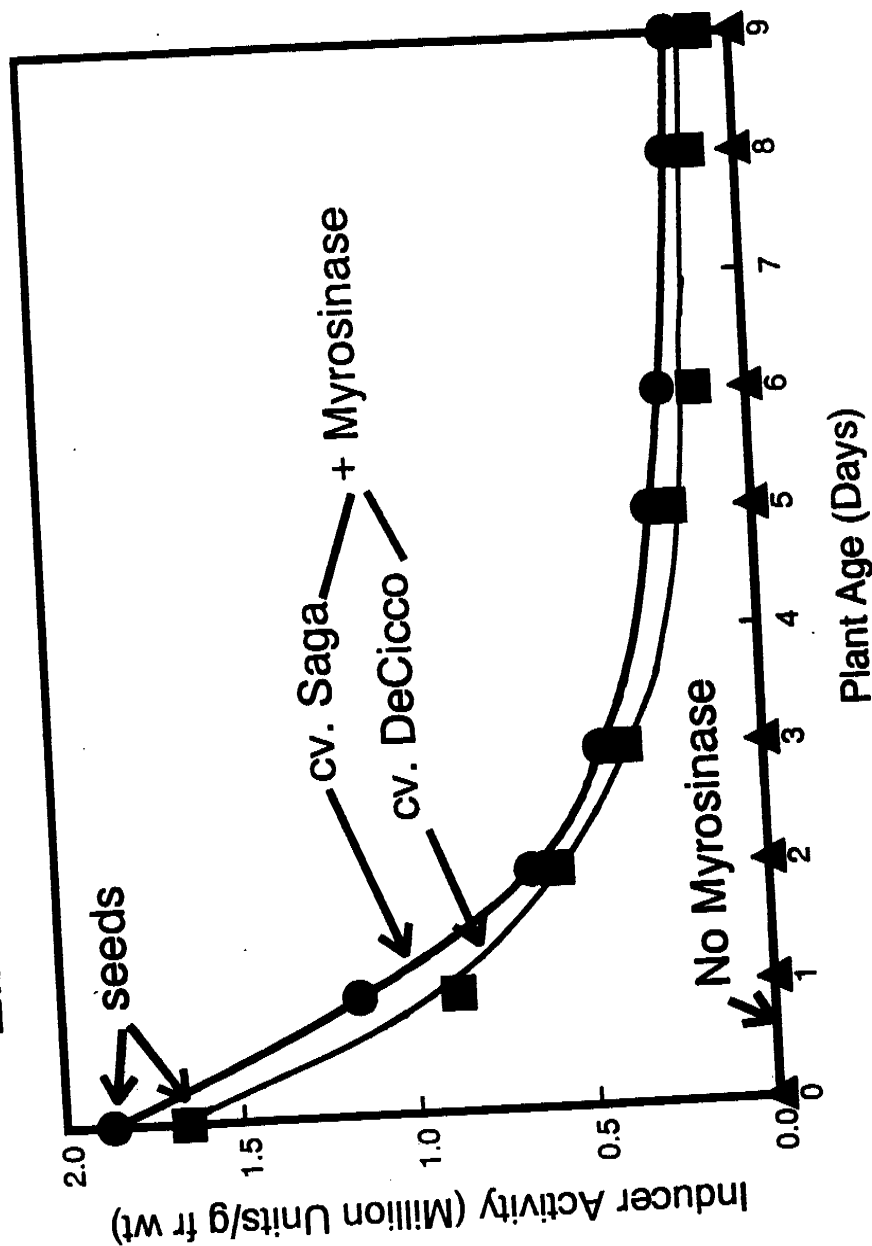


High Resolution NMR (600 MHz) in D_2O . Note: chirality of SO in Peak A induces multiplet for CH_2SO (Peak A), not observed for CH_2S (Peak C).

Figure 2

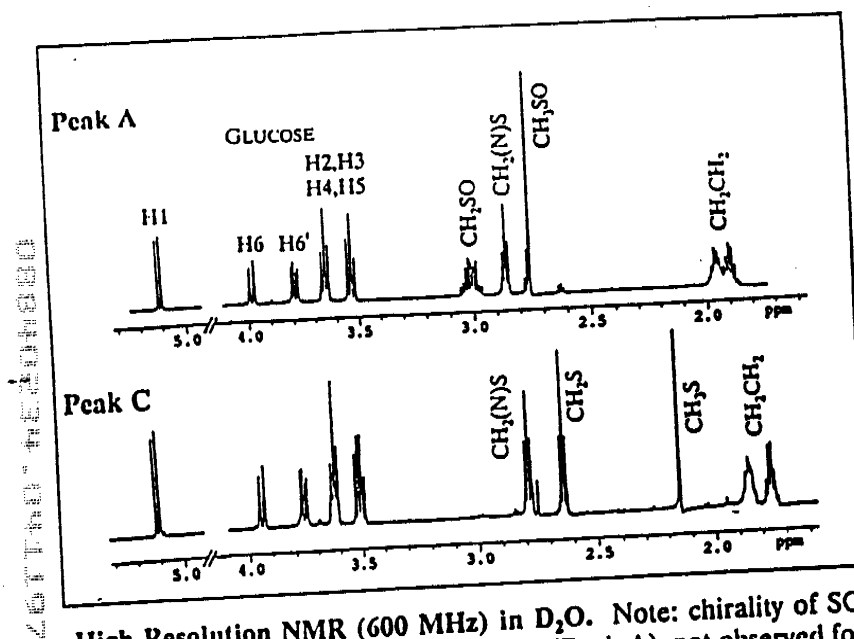
PRINTED DRAWINGS
AS ORIGINALLY FILED

Inducer Activity of Broccoli Sprouts Effect of Plant Age



▲ = < 1000 Units/g fr wt

Figure 1

PRINT OF DRAWINGS
AS ORIGINALLY FILED

High Resolution NMR (600 MHz) in D₂O. Note: chirality of SO in Peak A induces multiplet for CH₂SO (Peak A), not observed for CH₂S (Peak C).

Figure 2

2

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Paper Number

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Prior Application: Examiner: Art Unit: 1302

Attorney's Docket No.: 46528/109

ADDRESS TO:

Assistant Commissioner for Patents
Washington, D. C. 20231



#2/A

This is a request for filing a ☐ continuation,
☒ divisional application under 37 CFR 1.60 of prior application
Serial No. 08/528,858, filed on September 15, 1995, entitled
CANCER CHEMOPROTECTIVE FOOD PRODUCTS, by the following named
inventor(s): Jed W. FAHEY and Paul TALALAY.

1. ☒ Attached is a copy of the prior application, which the undersigned verifies is a true copy. (See 7 and 8 for drawing requirements.) (43 pages of specification, abstract, 47 claims, 2 sheets of drawings, copy of declaration.)
2. ☒ The filing fee is calculated below. (Small entity fees indicated in parentheses.)

CLAIMS AS FILED IN THE PRIOR APPLICATION
LESS ANY CLAIMS CANCELLED BY AMENDMENT BELOW

	<u>Number Filed</u>	<u>Number Extra</u>	<u>Rate</u>	<u>Basic Fee \$770 (\$385)</u>
Total Claims	31 - 20	11	x \$22 (x \$11)	121.00
Independent Claims	11 - 3	8	x \$80 (x \$40)	320.00
Multiple Dependent Claims			\$260	0.00
TOTAL FEE				\$826.00

3. ☒ The Commissioner is hereby authorized to charge any fees under 37 CFR 1.16 and 1.17 which may be required, or credit any overpayment to Deposit Account No. 19-0741.
4. ☒ A check in the amount of \$826.00 is enclosed.

Attorney's Docket No. 46528/109

5. [X] Cancel in this application original Claims 21-26, 28-34 and 36-38 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
6. [X] Amend the specification by inserting before the first line the following sentence:

Alt E2

~~This application is a [] continuation, [X] division, of application Serial No. 08/528,858, filed September 15, 1995.~~

08/528,858-044,137

7. [] Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate of this sheet is attached for filing in the prior application file. (May only be used if signed by a person authorized by Rule 138 and before payment of Base Issue Fee.)
8. [X] New drawings are attached.
- 9a. [] Priority of application Serial No. _____ filed on _____, in _____ is claimed under 35 USC 119.
- 9b. [] The certified copy of the priority document has been filed in prior application Serial No. _____, filed _____.
10. [X] The prior application is assigned of record to: Johns Hopkins School of Medicine
- 11a. [X] The power of attorney in the prior application is to: Bernhard D. Saxe, Reg. No. 28,665, and other members of the firm.
- 11b. [X] The power appears in the original papers in the prior application.
- 11c. [] Since the Power does not appear in the original papers, a copy of the power in the prior application is attached.
12. [X] Small entity status, based on the verified statement filed in parent application Serial No. 08/528,858, filed September 15, 1995, is claimed under 37 CFR 1.28(a).
13. [X] A Preliminary Amendment is attached. (Claims added by this amendment have been properly numbered consecu-

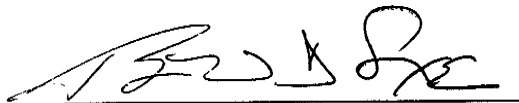
Attorney's Docket No. 46528/109

tively, beginning with the number next following the highest numbered original claim in the prior application.)

14. [X] Address all further communications to: (May only be completed by applicant, or attorney or agent of record.)

FOLEY & LARDNER
3000 K Street, N.W., Suite 500
P.O. Box 25696
Washington, D.C. 20007-8696
(Tel: 202/672-5300)

April 11, 1997
Date



Bernhard D. Saxe
Reg. No. 28,665

Attorney of Record

Address:

FOLEY & LARDNER
3000 K Street, N.W., Suite 500
P.O. Box 25696
Washington, D.C. 20007-8696
(Tel: 202/672-5300)

202/672-5300

3

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Paper Number

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#3/B

Attorney Docket No. 046528/0109

In re patent application of
Jed FAHEY et al.
Serial No. Unassigned
Filed: April 11, 1997
For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered into the application:

IN THE CLAIMS:

Please amend the claims as follows:

27. (Amended) A food product rich in glucosinolates made by the process [according to claim 21] comprising germinating cruciferous seeds, with the exception of cabbage, cress, mustard and radish seeds, and either harvesting sprouts prior to the 2-leaf stage, or harvesting at an earlier stage and promoting further maturation to reach said 2-leaf stage, to form a food product comprising a plurality of sprouts having at least 20,000 units per gram fresh weight of Phase 2 enzyme-inducing potential.

35. (Amended) A food product rich in glucosinolates, made by the process [according to claim 30] comprising germinating cruciferous seeds having at least 20,000 units per gram fresh weight of Phase 2

Attorney Docket No. 046528/0109

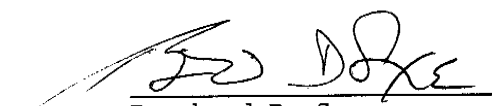
Dr and
enzyme-inducing potential and which contain non-toxic levels of indole glucosinolates and goitrogenic hydroxybutenyl glucosinolates, and either harvesting sprouts prior to the 2-leaf stage, or harvesting at an earlier stage and promoting further maturation to reach said 2-leaf stage, to form a food product comprising a plurality of sprouts having at least 20,000 units per gram fresh weight of Phase 2 enzyme-inducing potential.

REMARKS

Entry of the foregoing amendments prior to examination is respectfully requested.

Respectfully submitted,

April 11, 1997


Bernhard D. Saxe
Reg. No. 28,665

FOLEY & LARDNER
3000 K Street, N.W.
Suite 500
Washington, D.C. 20007-5109
Tel: (202) 672-5300

4

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Paper Number

4

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4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

046528/0109

In re patent application of

Jed FAHEY et al.

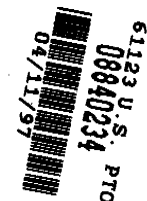
Serial No. Unassigned

Group Art Unit: Unassigned

Filed: April 11, 1997

Examiner:

For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS



#4

INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. § 1.56

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith on Form PTO-1449 is a listing of documents known to Applicants in order to comply with Applicants' duty of disclosure pursuant to 37 C.F.R. § 1.56.

The submission of any document herewith, which is not a statutory bar, is not intended as an admission that such document constitutes prior art against the claims of the present application or is considered to be material to patentability as defined in 37 C.F.R. § 1.56(b). Applicants do not waive any rights to take any action which would be appropriate to antedate or otherwise remove as a competent reference any document which is determined to be a prima facie prior art reference against the claims of the present application.

Serial No. Unassigned

CONCISE EXPLANATION OF
RELEVANCE OF EACH DOCUMENT

Applicants are submitting herewith on Form PTO-1449, a listing of the documents cited by or submitted to the Patent Office in parent application Serial No. 08/528,858, filed September 15, 1995. The relevance of these documents is explained in the parent application.

As provided in 37 C.F.R. §1.98(d), copies of the documents are not being provided since they were previously cited by or submitted to the Patent Office in parent application Serial No. 08/528,858 filed September 15, 1995.

Since this Information Disclosure Statement is being filed in compliance with 37 C.F.R. §1.97(b) within three (3) months of the filing date, no fee is required in connection with its filing.

Applicants respectfully request that the listed documents be considered by the Examiner and be made of

Serial No. Unassigned

record in the present application and that an initialled copy of Form PTO-1449 be returned in accordance with M.P.E.P. § 609.

Respectfully submitted,

April 11, 1997



Bernhard D. Saxe
Reg. No. 28,665

FOLEY & LARDNER
3000 K Street, N.W.
Suite 500
Washington, D.C. 20007-5109
(202) 672-5300

5

5

Paper Number

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5

5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 046528/0109

In re patent application of

Jed FAHEY et al.

Serial No. 08/840,234

Filed: April 11, 1997

For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

Group Art Unit: 1302

Examiner: UNKNOWN

DEC 24 1997

SECOND PRELIMINARY AMENDMENT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Preliminary to examination please amend the above-identified application as follows:

IN THE CLAIMS:

Kindly cancel claims 1- 20, 27, 35, and 39 - 47 without prejudice or disclaimer, and add the following claims:

--48. Cruciferous sprouts, with the exception of *Brassica oleracea capitata*, *Lepidium sativum*, *Sinapis alba*, *Sinapis nigra*, and *Raphanus sativus* sprouts, harvested between the onset of germination up to and including the 2-leaf stage.

49. The cruciferous sprouts according to claim 48, wherein said sprouts are harvested 1 to 14 days post-germination and have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

50. The cruciferous sprouts according to claim 48, wherein said sprouts are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*,

Serial No. 08/840,234

gemmifera, gongylodes, italica, medullosa, palmifolia, ramosa, sabauda, sabellica, and selensia.

51. The cruciferous sprouts according to claim 50, wherein said sprouts are a *Brassica oleracea* variety *italica*.

52. The cruciferous sprouts according to claim 50, wherein said sprouts are a *Brassica oleracea* variety *botrytis*.

53. The cruciferous sprouts according to claim 52, wherein said sprouts are a *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

54. The cruciferous sprouts according to claim 48, wherein said sprouts are substantially free of Phase 1 enzyme-inducing potential.

C 55. The cruciferous sprouts according to claim 54, wherein the ratio of monofunctional to bifunctional Phase 2 enzyme inducers is at least 20 to 1.

56. A non-toxic solvent extract of the cruciferous sprouts according to claim 48.

57. The non-toxic solvent extract according to claim 56, wherein said solvent is water.

58. The non-toxic solvent extract according to claim 57, further comprising a cruciferous vegetable comprising an active myrosinase enzyme.

59. The non-toxic solvent extract according to claim 58, wherein said cruciferous vegetable is of the genus *Raphanus*.

Serial No. 08/840,234

60. A food product comprising the cruciferous sprouts according to claim 48, optionally including cruciferous seeds; extracts of said sprouts or seeds; or any combination of said sprouts, seeds or extracts.

61. The food product according to claim 60, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts.

62. A food product made by the process of germinating cruciferous seeds, with the exception of *Brassica oleracea capitata*, *Lepidium sativum*, *Sinapis alba*, *Sinapis nigra*, and *Raphanus sativus* sprouts, and harvesting sprouts between the onset of germination up to and including the 2-leaf stage, to form a food product comprising a plurality of sprouts.

63. The food product according to claim 62, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates and are harvested 1 to 14 days post-germination.

64. The food product according to claim 63, wherein said sprouts are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemnifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

65. The food product according to claim 64, wherein said sprouts are *Brassica oleracea* variety *italica*.

Serial No. 08/840,234

66. The food product according to claim 64, wherein said sprouts are *Brassica oleracea* variety *botrytis*.

67. The food product according to claim 66, wherein said sprouts are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

68. The food product according to claim 62, wherein said sprouts are substantially free of Phase 1 enzyme-inducing potential.--

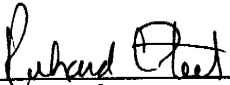
REMARKS

Claims 48-68 are now pending. Claims 1-20, 27, 35 and 39-47 have been canceled. New claims 48-68 have been added. Support for the new claims can be found in the original claims and the specification as filed. Specifically, see Page 8, line 33 and page 14, line 7.

Respectfully submitted,

December 24, 1997
Date

FOLEY & LARDNER
Suite 500
3000 K Street, N.W.
Washington, DC 20007-5109
(202) 672-5300


Richard C. Peet
Registration No. 35,792

6

6

Paper Number

6

6

6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 046528/0109/JOHN

In re patent application of

Jed FAHEY et al.

Serial No. 08/840,234

Filed: April 11, 1997

For: CANCER CHEMOPROTECTIVE FOOD PRODUCTS

Group Art Unit: 1302

Examiner: L. Wong

*Revised D
3-31-98
dw
#6*

THIRD PRELIMINARY AMENDMENT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

FEB 25 1998
GROUP 1100

Sir:

Preliminary to examination please amend the above-identified application as follows:

IN THE CLAIMS:

Kindly cancel claims 48-68 without prejudice or disclaimer and add the following claims:

~~1-68~~ 1. A method of preparing a human food product comprising cruciferous sprouts containing high Phase 2 enzyme-inducing potential and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, comprising the steps of:

(a) identifying seeds which produce said sprouts, with the exception of *Brassica oleracea capitata*, *Lepidium sativum*, *Sinapis alba*, *Sinapis nigra*, and *Raphanus sativus* sprouts;

\$1

(b) germinating said seeds; and

(c) harvesting said sprouts between the onset of germination up to and including the 2-leaf stage, to form a human food product comprising a plurality of said sprouts.

2
~~70~~ 2. The method according to claim ~~69~~ 1, wherein said sprouts are harvested 1 to 14 days post-germination and contain at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing

46

Application Serial No. 08/840,234

potential when measured after 3-days of growth and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

³ 71. The method according to claim ¹ ~~69~~ or ² ~~70~~, wherein said seeds are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemnifera*, *gongyloides*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

⁴ 72. The method according to claim ³ ~~71~~, wherein said seeds are *Brassica oleracea* variety *italica*.

⁵ 73. The method according to claim ³ ~~71~~, wherein said seeds are *Brassica oleracea* variety *botrytis*.

⁶ 74. The method according to claim ⁵ ~~73~~, wherein said seeds are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

⁷ 75: The method according to claim ¹ ~~69~~, wherein said sprouts are substantially free of Phase 1 enzyme-inducing potential.

⁸ 76. A human food product comprising cruciferous sprouts made according to the method of claim ~~69~~.

⁹ 77. A method of preparing a human food product, comprising extracting glucosinolates and isothiocyanates from cruciferous sprouts, ^{rich in glucosinolates} with the exception of *Brassica oleracea capitata*, *Lepidium sativum*, *Sinapis alba*, *Sinapis nigra*, and *Raphanus sativus* sprouts, harvested between the onset of germination up to and including the 2-leaf stage, or from cruciferous seeds, or a combination thereof, with a non-toxic solvent, removing the extracted sprouts, seeds, or a combination thereof from said

Application Serial No. 08/840,234

solvent and, and recovering the extracted glucosinolates and isothiocyanates.

¹⁹
78. The method according to claim ⁹77, wherein the sprouts are harvested 1 to 14 days post-germination and contain at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.

¹¹
79. The method according to claim ¹⁰78, wherein said seeds are a *Brassica oleracea* selected from the group of varieties consisting of *acephala*, *alboglabra*, *botrytis*, *costata*, *gemnifera*, *gongylodes*, *italica*, *medullosa*, *palmifolia*, *ramosa*, *sabauda*, *sabellica*, and *selensia*.

01 ¹²
80. The method according to claim ¹¹79, wherein said seeds are *Brassica oleracea* variety *italica*.

¹³
81. The method according to claim ¹¹79, wherein said seeds are *Brassica oleracea* variety *botrytis*.

¹⁴
82. The method according to claim ¹³81, wherein said seeds are *Brassica oleracea* variety *botrytis* subvariety *cauliflora*.

¹⁵
83. The method according to claim ⁹77, wherein said sprouts are substantially free of Phase 1 enzyme-inducing potential.

¹⁶
84. The method according to claim ⁹77, further comprising the step of drying said extracted glucosinolates and isothiocyanates.

Application Serial No. 08/840,234

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85. The method according to claim 9, wherein active myrosinase enzyme is mixed with said cruciferous sprouts, said cruciferous seeds, said extracted glucosinolates and isothiocyanates, or a combination thereof.

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86. An extract prepared according to the method of any one of claims 7, 84 or 85.--

REMARKS

Claims 69-86 are now pending. Claims 1-68 have been canceled. New claims 69-86 have been added. Support for the new claims can be found in the original claims and the specification as file.

Attached for the Examiner's convenience are copies of all references cited in the Information Disclosure Statement submitted on April 11, 1997, since Examiner cannot easily access the parent file.

Respectfully submitted,

February 25, 1998
Date

FOLEY & LARDNER
Suite 500
3000 K Street, N.W.
Washington, DC 20007-5109
(202) 672-5300

Richard C. Peet
Richard C. Peet
Registration No. 35,792